(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 895 082 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
03.02.1999 Bulletin 1999/05

(21) Application number: **98306107.8**

(22) Date of filing: **31.07.1998**

(51) Int Cl.[6]: **G01N 33/543**, C12Q 1/68, C07K 17/14, C12N 11/14, G01N 33/53, G01N 33/68

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **01.08.1997 JP 207837/97**
20.10.1997 JP 287046/97
24.07.1998 JP 209923/98

(71) Applicant: **CANON KABUSHIKI KAISHA**
Tokyo (JP)

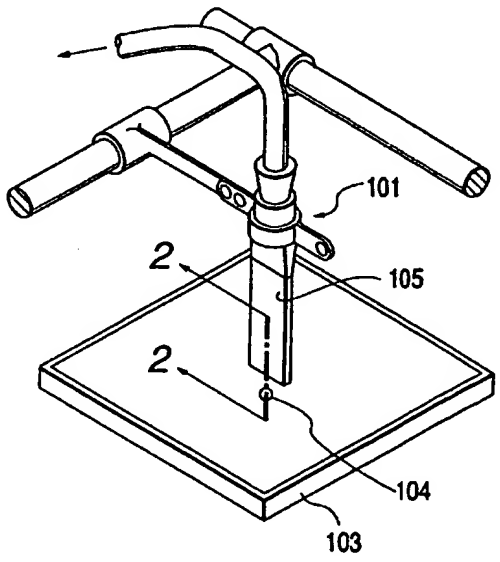
(72) Inventors:
- **Okamoto, Tadashi**
  Ohta-ku, Tokyo (JP)
- **Yamamoto, Nobuko**
  Ohta-ku, Tokyo (JP)
- **Suzuki, Tomohiro**
  Ohta-ku, Tokyo (JP)

(74) Representative:
Beresford, Keith Denis Lewis et al
BERESFORD & Co.
2-5 Warwick Court
High Holborn
London WC1R 5DJ (GB)

(54) Method of spotting probe on solid support, probe array and method of manufacturing thereof, and method of detecting target substance and method of identifying structure of target substance using probe array

(57) Provided is a method of spotting a probe densely and efficiently on a surface of a solid support. A liquid containing a probe is attached to a solid support as droplets to form spots containing the probe on the solid support by an ink jet method.

FIG. 1



EP 0 895 082 A2

Printed by Jouve, 75001 PARIS (I

Description

Background of the Invention

Field of the Invention

[0001] The present invention relates to a method of spotting a probe on a solid support, a probe array and a method of manufacturing thereof, and a method of detecting a target single-stranded (ss) nucleic acid and a method of identifying a base sequence of a target ss nucleic acid using the probe array.

Related Background Art

[0002] As a method to determine a base sequence of a nucleic acid, detect a target nucleic acid in a sample, and identify various bacteria swiftly and accurately, proposed is the use of a probe array where one or more substances which can bind specifically to a target nucleic acid, so-called probes, are arranged on a solid support at a large number of sites. As a general method of manufacturing such probe arrays as described in EP No. 0373203B1, (1) the nucleic acid probe is synthesized on a solid support or (2) a previously synthesized probe is supplied onto a solid support. USP No. 5405783 discloses the method (1) in detail. Concerning the method (2), USP No. 5601980 and Science Vol. 270, p. 467 (1995) teach a method of arranging cDNA in an array by using a micropipet.

[0003] In the above method (1), it is not necessary to synthesize a nucleic acid probe in advance, since the nucleic acid probe is synthesized directly on a solid support. However, it is difficult to purify a probe nucleic acid synthesized on a solid support. The accuracy in determining the base sequence of a nucleic acid and in the detection of a target nucleic acid in a sample using a probe array largely depends on the correctness of the base sequence of the nucleic acid probe. For the method (1), therefore, further improvement in accuracy of a nucleic acid probe is required in order to manufacture a probe array of higher quality. In the method (2), a step of synthesizing a nucleic acid probe is required prior to the fixation of the nucleic acid probe on a solid support, but the nucleic acid probe can be purified before binding the probe to a solid support. For this reason, presently, the method (2) is considered to be more preferable than the method (1) as a method of manufacturing a probe array of high quality. However, the method (2) has a problem in the method of spotting a nucleic acid probe densely on a solid support. For example, when a probe array is used to determine the base sequence of a nucleic acid, it is preferable to arrange as many kinds of nucleic acid probes as possible on a solid support. When mutations in a gene are to be detected efficiently, it is preferable to arrange nucleic acid probes of sequences corresponding to the respective mutations on a solid support. In addition, when a target nucleic acid in a sample or to gene mutations and deletions are detected, it is desirable that the amount of the sample taken from a subject, specifically a blood sample, is as small as possible. Thus, it is preferable that as much information as possible on the base sequence is obtained using a small sample amount. Considering these points, it is preferable that, for example, 10,000 or more nucleic acid probe spots per square inch are arranged in a probe array.

SUMMARY OF THE INVENTION

[0004] As the result of the research carried out by the inventors to solve above-discussed problems, they have found that an ink jet ejection method enables spotting of a probe in a markedly high density and achieved the present invention.

[0005] It is an object of the present invention to provide a method of spotting an extremely small amount of probe efficiently and accurately on a solid support without damaging the probe.

[0006] It is another object of the present invention to provide a probe array that can provide more information on nucleic acid more accurately even using a small amount of sample.

[0007] It is still another object of the present invention to provide a method of efficiently manufacturing a probe array, in which a large number of probes are bound to a solid support, without damaging the probes.

[0008] It is further another object of the present invention to provide a method of efficiently detecting a target substance that may be contained in a sample.

[0009] It is still other object of the present invention to provide a method of identifying the structure of a target substance to obtain information on the structure of the target substance even from a small amount of sample.

[0010] According to one aspect of the present invention, there provided is a method of spotting a probe which can bind specifically to a target to a solid support. The method comprises a step of supplying a liquid containing a probe on a surface of a solid support by an ink jet method and adhering the liquid on the surface of the solid support. The use of the spotting method according to the above embodiment allows accurate and efficient provision of a probe on a solid support and efficient manufacturing of a probe array.

[0011] According to another aspect of the present invention, provided is a probe array comprised of a plurality of spots of a probe, where the spots are provided independently at a plurality of sites of the surfac of a solid support in

STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULAR TYPE: Oth r nucl ic acid: Synthetic DNA ADDITIONAL INFORMATION: Thi l gr up bound at th 5'-terminus
SEQUENCE DESCRIPTION

TGTATAACCACGCCCAGT

Claims

1. A method of spotting a probe which can bind specifically to a target to a solid support comprising the steps of:

   supplying a liquid containing a probe on a surface of a solid support by an ink jet method and attaching the liquid, and
   forming a spot of probe on the surface of the solid support.

2. The method of spotting according to claim 1 wherein the probe is a single-stranded nucleic acid probe.

3. The method of spotting according to claim 2 wherein the single-stranded nucleic acid probe includes a single-stranded DNA probe.

4. The method of spotting according to claim 2 wherein the single-stranded nucleic acid probe includes an RNA probe.

5. The method of spotting according to claim 2 wherein the single-stranded nucleic acid probe includes a single-stranded PNA probe.

6. The method of spotting according to claim 2 wherein the surface of the solid support has a first functional group and the single-stranded nucleic acid probe has a second functional group, and the first and second functional groups react each other by contact.

7. The method of spotting according to claim 6 wherein the first functional group on the surface of the solid support is a maleimido group and the second functional group of the single-stranded nucleic acid probe is a thiol (SH) group.

8. The method of spotting according to claim 7 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with N-(6-maleimidocaproyloxy) succinimide.

9. The method of spotting according to claim 7 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with succinimidyl-4-(maleimido phenyl) butyrate.

10. The method of spotting according to claim 7 wherein the maleimido group is reacted with the thiol group for at least 30 minutes.

11. The method of spotting according to claim 10 wherein the single-stranded nucleic acid comprises a single-stranded PNA probe, at the terminus of which the thiol group exists, and the maleimido group is reacted with the thiol group for at least 2 hours.

12. The method of spotting according to claim 11 wherein the thiol group at the terminus of the single-stranded PNA probe is introduced by binding cysteine group to the N-terminus of the single-stranded PNA probe.

13. The method of spotting according to claim 6 wherein the first functional group on the surface of the solid support is an epoxy group and the second functional group of the single-stranded nucleic acid probe is an amino group.

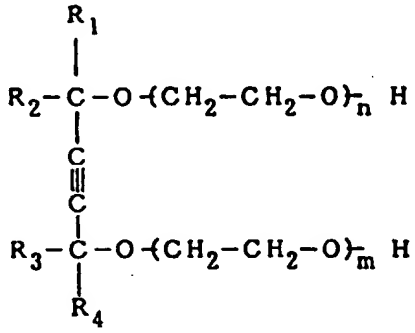
14. The method of spotting according to claim 13 wherein the solid support is a glass plate and the epoxy group is

introduced by applying a silane compound having an epoxy group in the molecule th reof n the surface f the glass plate and reacting the compound with the glass plate.

15. The method f spotting according to claim 13 wherein the epoxy gr up is introduced by applying polyglycidyl methacrylat having an epoxy group on the solid support.

16. The method of spotting according to claim 1 wherein the liquid contains urea at 5-10 wt%, glycerin at 5-10 wt%, thiodiglycol at 5-10 wt%, and an acetylene alcohol at 1 wt% of the liquid.

17. The method of spotting according to claim 16 wherein the acetylene alcohol has a structure represented by the following general formula (I):



(I)

(wherein, R_1, R_2, R_3, and R_4 represent an alkyl group, each m and n represent an integral, and m=0 and n=0 or $1 \leq m+n \leq 30$, and when m+n=1, m or n is 0.)

18. The method of spotting according to claim 2 wherein a concentration of the single-stranded nucleic acid probe in the liquid is 0.05-500 μM.

19. The method of spotting according to claim 18 wherein a concentration of the single-stranded nucleic acid probe in the liquid is 2-50 μM.

20. The method of spotting according to claim 2 wherein a length of the single-stranded nucleic acid probe is 2-5,000 bases.

21. The method of spotting according to claim 20 wherein a length of the single-stranded nucleic acid probe is 2-60 bases.

22. The method of spotting according to claim 1 wherein the ink jet method is a bubble jet method.

23. The method of spotting according to claim 1 wherein the probe is an oligopeptide or a polypeptide with a specific amino acid sequence.

24. The method of spotting according to claim 1 wherein the probe is a protein.

25. The method of spotting according to claim 24 wherein the protein is an antibody.

26. The method of spotting according to claim 24 wherein the protein is an enzyme.

27. The method of spotting according to claim 1 wherein the probe is an enzyme.

28. The method of spotting according to claim 1 wherein the liquid is supplied so as to form independent spots in a density of 10,000 spots per square inch on the solid support.

29. The method of spotting according to claim 1 wherein the solid support has a flat surface and homogenous surface properties.

30. The method of spotting according to claim 29 wherein the liquid is supplied on the surface of the solid support so as to obtain a distance between the adjacent spots not smaller than the maximum width of the spot.

31. The method of spotting according to claim 30 wherein blocking is performed on the surface of the solid support to prevent a sample from attaching to the surface other than spots of the surface of the solid support.

32. The method of spotting according to claim 31 wherein the blocking is achieved by using bovine serum albumin.

33. The method of spotting according to claim 1 wherein the solid support is partitioned by a matrix arranged in a pattern on the surface, a plurality of wells whose bottom is the surface of the solid support exposed in the pattern are provided, and the liquid is supplied to the respective wells.

34. The method of spotting according to claim 33 wherein the solid support is optically transparent and the matrix is opaque.

35. The method of spotting according to claim 33 wherein the matrix comprises a resin.

36. The method of spotting according to claim 33 wherein the surface of the matrix is hydrophobic.

37. The method of spotting according to claim 33 wherein the bottom of the wells is hydrophilic.

38. The method of spotting according to claim 33 wherein the matrix has a thickness of 1-20 μm.

39. The method of spotting according to claim 33 wherein the wells have a maximum width of 200 μm.

40. The method of spotting according to claim 33 wherein the matrix has a width 1/2 - 2 times the maximum width of the wells.

41. A probe array comprising a plurality of spots of a probe, the spots being provided independently at a plurality of sites of a surface of a solid support in a density of 10,000 spots per square inch or higher.

42. The probe array according to claim 41 wherein the solid support has a flat surface and homogenous surface properties.

43. The probe array according to claim 42 wherein the probe is a single-stranded nucleic acid probe.

44. The probe array according to claim 43 wherein the single-stranded nucleic acid probe includes a single-stranded DNA probe.

45. The probe array according to claim 43 wherein the single-stranded nucleic acid includes a single-stranded RNA probe.

46. The probe array according to claim 43 wherein the single-stranded nucleic acid includes a single-stranded PNA probe.

47. The probe array according to claim 43 wherein the single-stranded nucleic acid is covalently bound to the surface of the solid support by a reaction between a first functional group on the surface of the solid surface and a second functional group of the single-stranded nucleic acid probe.

48. The probe array according to claim 47 wherein the first functional group on the surface of the solid support is a maleimido group and the second functional group of the single-stranded nucleic acid probe is a thiol (SH) group.

49. The probe array according to claim 48 wherein the single-stranded nucleic acid probe is a single-stranded PNA probe and contains a cysteine residue on an N-terminus side.

50. The probe array according to claim 47 wherein the first functional group on the surface of the solid support is an epoxy group and the second functional group of the single-stranded nucleic acid probe is an amino group.

51. The probe array according to claim 42 wher in the spots are formed by supplying a liquid containing the prob on the solid support.

52. The probe array according to claim 42 wherein the prob is an oligopeptide or a polypeptide with a specific amin acid sequence.

53. The probe array according to claim 42 wherein the probe is a protein.

54. The probe array according to claim 53 wherein the protein is an antibody.

55. The probe array according to claim 53 wherein the protein is an enzyme.

56. The probe array according to claim 42 wherein the probe is an antigen.

57. The probe array according to claim 42 wherein a distance between the adjacent spots is not smaller than a maximum width of the spot.

58. The probe array according to claim 41 wherein the solid support is partitioned by a matrix arranged in a pattern on the surface, a plurality of wells whose bottom is the surface of the solid support exposed in a pattern are provided, and the liquid is supplied to the respective wells.

59. The probe array according to claim 58 wherein the probe is a single-stranded nucleic acid probe.

60. The probe array according to claim 59 wherein the single-stranded nucleic acid probe includes a single-stranded DNA probe.

61. The probe array according to claim 59 wherein the single-stranded nucleic acid includes a RNA probe.

62. The probe array according to claim 59 wherein the single-stranded nucleic acid includes a single-stranded PNA probe.

63. The probe array according to claim 62 wherein the single-stranded nucleic acid is covalently bound to the surface of the solid support by a reaction between the first functional group of the surface of the solid surface and the second functional group on the single-stranded nucleic acid probe.

64. The probe array according to claim 63 wherein the first functional group on the surface of the solid support is a maleimido group and the second functional group of the single-stranded nucleic acid probe is a thiol (SH) group.

65. The probe array according to claim 64 wherein the single-stranded nucleic acid probe is a single-stranded PNA probe and contains a cysteine residue on an N-terminal side.

66. The probe array according to claim 63 wherein the first functional group on the surface of the solid support is an epoxy group and the second functional group of the single-stranded nucleic acid probe is an amino group.

67. The probe array according to claim 58 wherein the spots are formed by supplying a liquid containing a probe on the solid support.

68. The probe array according to claim 58 wherein the probe is an oligopeptide or a polypeptide with a specific amino acid sequence.

69. The probe array according to claim 58 wherein the probe is a protein.

70. The probe array according to claim 69 wherein the protein is an antibody.

71. The probe array according to claim 69 wherein the protein is an enzyme.

72. The probe array according to claim 58 wherein the probe is an antigen.

73. The probe array according to claim 58 wherein the matrix is paque.

74. The probe array according to claim 73 wherein the solid support is optically transparent.

75. The probe array according to claim 58 wherein the matrix comprises a resin.

76. The probe array according to claim 58 wherein the probe is attached only to the wells.

77. The probe array according to claim 58 wherein the matrix has a thickness of 1-20 μm.

78. The probe array according to claim 58 wherein the wells have a maximum width of 200 μm.

79. The probe array according to claim 58 wherein a distance between the wells is 1/2 - 2 times the maximum width of the wells.

80. The probe array according to claim 41 wherein the probe array comprises at least 2 spots each of which comprises a different kind of probe.

81. A method of manufacturing a probe array having a plurality of spots arranged independently in a plurality of sites on a surface of a solid support, the spots containing a probe which can bind specifically to a target substance comprising a step of supplying a liquid containing the probe and attaching the liquid to a predetermined site on the surface of the solid support by means of an ink jet method to form the spots.

82. The method of manufacturing according to claim 81 wherein the probe is a single-stranded nucleic acid probe.

83. The method of manufacturing according to claim 82 wherein the single-stranded nucleic acid probe is a single-stranded DNA probe.

84. The method of manufacturing according to claim 82 wherein the single-stranded nucleic acid probe is an RNA probe.

85. The method of manufacturing according to claim 82 wherein the single-stranded nucleic acid probe is a single-stranded PNA probe.

86. The method of manufacturing according to claim 82 wherein the surface of the solid surface has a first functional group and the single-stranded nucleic acid probe has a second functional group, and the first and the second functional groups react each other by contact.

87. The method of manufacturing according to claim 86 wherein the first functional group on the surface of the solid support is a maleimido group and the second functional group of the single-stranded nucleic acid probe is a thiol (SH) group.

88. The method of manufacturing according to claim 87 wherein the solid support is a glass plate and a maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with N-(6-maleimidocaproyloxy) succinimide.

89. The method of manufacturing according to claim 87 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with succinimidyl-4-(maleimido phenyl) butyrate.

90. The method of manufacturing according to claim 87 wherein the maleimido group is reacted with the thiol group for at least 30 minutes.

91. The method of manufacturing according to claim 90 wherein the single-stranded nucleic acid is a single-stranded PNA probe, at the terminus of which the thiol group exists, the maleimido group is reacted with the thiol group for at least 2 hours.

92. The method of manufacturing according to claim 91 wherein the thiol group at the terminus of the single-stranded

PNA probe is introduced by binding cysteine group to an N-terminal of th single-stranded PNA probe.

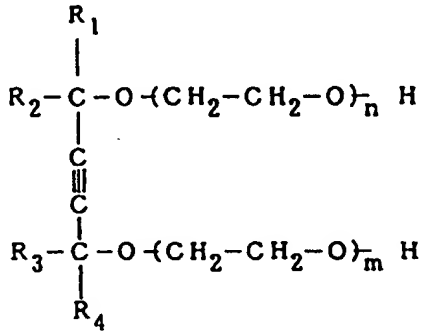
93. The method of manufacturing according to claim 86 wherein the first functional gr up on the surface of the solid support is an epoxy group and the second functional group of the single-stranded nucleic acid probe has is an amino group.

94. The method of manufacturing according to claim 93 wherein the solid support is a glass plate and the epoxy group is introduced by applying a silane compound having an epoxy group in the molecule thereof on the surface of the glass plate and reacting the compound with the glass plate.

95. The method of manufacturing according to claim 93 wherein the epoxy group is introduced by applying polyglycidyl methacrylate having an epoxy group on the solid support.

96. The method of manufacturing according to claim 93 wherein the liquid contains urea at 5-10 wt%, glycerin at 5-10 wt%, thiodiglycol at 5-10 wt%, and an acetylene alcohol at 1 wt% of the liquid.

97. The method of manufacturing according to claim 96 wherein the acetylene alcohol has a structure represented by the following general formula (I):



(I)

(wherein, R_1, R_2, R_3, and R_4 represent an alkyl group, each m and n represent an integral, and m=0 and n=0, or $1 \leq m+n \leq 30$, and when m+n=1, m or n is 0.)

98. The method of manufacturing according to claim 82 wherein a concentration of the single-stranded nucleic acid probe in the liquid is 0.05-500 μM.

99. The method of manufacturing according to claim 98 wherein the concentration of the single-stranded nucleic acid probe in the liquid is 2-50 μM.

100. The method of manufacturing according to claim 82 wherein a length of the single-stranded nucleic acid probe is 2-5,000 bases.

101. The method of manufacturing according to claim 100 wherein the length of the single-stranded nucleic acid probe is 2-60 bases.

102. The method of manufacturing according to claim 81 wherein the ink jet method is a bubble jet method.

103. The method of manufacturing according to claim 81 wherein the liquid is supplied so as to form independent spots in a density of 10,000 spots per square inch on the solid support of higher.

104. The method of manufacturing according to claim 81 wherein the probe is an oligopeptide or a polypeptide with a specific amino acid sequence.

105. The method of manufacturing according to claim 81 wherein the probe is a protein.

106. The method of manufacturing according t claim 105 wherein the protein is an antibody.

107. The method of manufacturing acc rding t claim 105 wherein the protein is an nzym .

108. The method of manufacturing according to claim 81 wherein the probe is an antigen.

109. The method of manufacturing according to claim 81 wherein the solid support has a flat surface and homogenous surface properties.

110. The method of manufacturing according to claim 109 wherein blocking is performed, following spotting of the probe to the solid support, to prevent a sample from attaching to the surface other than the spots.

111. The method of manufacturing according to claim 110 wherein the blocking comprises a step of immersing the solid support to which the spots have been formed in an aqueous solution of bovine serum albumin.

112. The method of manufacturing according to claim 111 wherein a concentration of the aqueous solution of bovine serum albumin is 0.1-5%.

113. The method of manufacturing according to claim 111 wherein the solid support is immersed in an aqueous solution of bovine serum albumin for at least 2 hours.

114. The method of manufacturing according to claim 109 wherein the liquid is supplied on the surface of the solid support so as to obtain a distance between the adjacent spots not smaller than a maximum width of the spot.

115. The method of manufacturing according to claim 81 wherein the solid support is partitioned by a matrix arranged in a pattern on the surface, a plurality of wells whose bottom is the surface of the solid support exposed in a pattern are provided, and the liquid is supplied to the respective wells.

116. The method of manufacturing according to claim 115 wherein the solid support is optically transparent and the matrix is opaque.

117. The method of manufacturing according to claim 115 wherein the matrix comprises a resin.

118. The method of manufacturing according to claim 115 wherein the surface of the matrix is hydrophobic.

119. The method of manufacturing according to claim 115 wherein the bottom of the wells is hydrophilic.

120. The method of manufacturing according to claim 115 wherein the wells have a maximum width of 200 μm.

121. The method of manufacturing according to claim 115 wherein the matrix has a width 1/2 - 2 times the maximum width of the wells.

122. The method of manufacturing according to claim 115 wherein a thickness of the matrix is 1-20 μm.

123. The method of manufacturing according to claim 115 wherein the matrix pattern is formed by photolithography.

124. The method of manufacturing according to claim 123 wherein the photolithography comprises a step of forming a resin layer on the surface of the solid support, forming a photoresist layer on the resin layer, exposing the photoresist layer to light in a pattern corresponding to the matrix pattern, and developing to form the pattern of the photoresist on the resin layer; and a step of patterning the resin layer using the pattern of the photoresist as a mask and then removing the pattern of the photoresists.

125. The method of manufacturing according to claim 123 wherein the photolithography comprises the steps of forming a photosensitive resin layer on the surface of the solid support, exposing the photosensitive resin layer to light in a pattern corresponding to the matrix pattern, and developing.

126. The method of manufacturing according to claim 125 wherein the photosensitive resin layer is select d from the group consisted of a UV resist, a DEEP-UV resist, or an ultraviolet cure r sin.

127. The method of manufacturing according to claim 126 wher in the UV resist is selected from the group consisted of a cyclized polyisoprene-aromatic bisazide resist, a phen l resin-aromatic azide compound r sist, r a novolak resin-diazonaphtoquinone resist.

128. The method of manufacturing according to claim 126 wherein th DEEP-UV resin is a radiolysable polymer resist or a dissolution suppressant resist.

129. The method of manufacturing according to claim 128 wherein the radiation decomposition polymer resist is at least one selected from a group consisting of polymethyl methacrylate, polymethylene sulfone, polyhexafluorobutyl methacrylate, polymethylisopropenyl ketone, and poly-l-trimethylsilyl propyne bromide.

130. The method of manufacturing according to claim 128 wherein the dissolution suppressant resist is o-nitrobenzyl cholate ester.

131. The method of manufacturing according to claim 126 wherein the DEEP-UV resist is polyvinylphenol-3,3'-diazidediphenyl sulfone or polyglycidyl polymethacrylate.

132. The method of manufacturing according to claim 125 wherein water repellency of the matrix pattern formed by patterning of the photosensitive resin layer is further improved by postbaking of the matrix pattern.

133. The method of manufacturing according to claim 115 wherein a first functional group which can form a covalent bond with a second functional group of the probe is introduced on the surface of the solid support prior to formation of the wells.

134. The method of manufacturing according to claim 115 wherein a first functional group which can form a covalent bond with a second functional group of the probe is introduced on the surface of the solid support following formation of the wells.

135. The method of manufacturing according to claim 134 wherein a solution containing a compound for introducing the first functional group to the surface of the solid support is supplied to the wells.

136. The method of manufacturing according to claim 135 wherein the solution is supplied to the wells by means of the ink jet method.

137. The method of manufacturing according to claim 136 wherein the solution is a silane coupling agent containing a silane compound having an epoxy group or an amino group in its molecule.

138. The method of manufacturing according to claim 136 wherein the solution contains a compound which can react with an amino group on a glass substrate to introduce a maleimido group on the glass substrate.

139. The method of manufacturing according to claim 138 wherein the compound is N-maleimidocaproyloxy succinimide or succinimidyl-4-(maleimidophenyl) butyrate.

140. A method for detecting whether a target substance is contained in a sample, comprising the steps of:

providing a probe array comprising a plurality of spots each containing a probe which specifically binds to the target substance, the spots being arranged independently on a solid support;
contacting the sample with each of the spots; and
detecting presence or absence of a reacted product between the target substance and the probe, wherein the respective spots are formed by spotting a liquid containing the probe on the solid support by an ink jet method.

141. The method according to claim 140 wherein the target substance is a single-stranded nucleic acid having a first base sequence and the probe is a single-stranded nucleic acid probe having a second base sequence complementary to the first base sequence.

142. Th method according to claim 141 wherein th single-stranded nucleic acid probe is a single-stranded DNA probe.

143. The method according to claim 141 wherein the single-stranded nucleic acid probe is an RNA probe.

144. The method according to claim 141 wher in the single-stranded nucleic acid probe is a single-stranded PNA probe.

145. The method according to claim 141 wherein th surfac of th solid surfac has a first functional gr up and the single-stranded nucleic acid probe has a second functional gr up, respectively, and the functional groups react each other by contact.

146. The method according to claim 145 wherein the first functional group on the surface of the solid support is a maleimido group and the second functional group of the single-stranded nucleic acid probe is a thiol (SH) group.

147. The method according to claim 146 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with N-(6-maleimidocaproyloxy) succinimide.

148. The method according to claim 146 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with succinimidyl-4-(maleimidophenyl) butyrate.

149. The method according to claim 146 wherein the maleimido group is reacted with the thiol group for at least 30 minutes.

150. The method according to claim 149 wherein the single-stranded nucleic acid comprises a single-stranded PNA probe having a thiol group on the terminus and the maleimido group is reacted with the thiol group for at least 2 hours.

151. The method according to claim 146 wherein the thiol group at a terminus of the single-stranded PNA probe is introduced by binding cysteine group to an N-terminus of the single-stranded PNA probe.

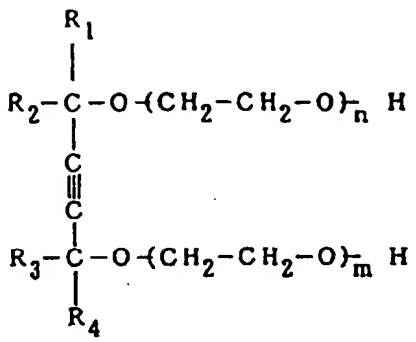
152. The method according to claim 145 wherein the first functional group on the surface of the solid support is an epoxy group and the second functional group of the single-stranded nucleic acid probe is an amino group.

153. The method according to claim 152 wherein the solid support is a glass plate and the epoxy group is introduced by applying a silane compound having an epoxy group in the molecule thereof on the surface of the glass plate and reacting the compound with the glass plate.

154. The method according to claim 152 wherein the epoxy group is introduced by applying polyglycidyl methacrylate having an epoxy group on the solid support.

155. The method according to claim 141 wherein the liquid contains urea at 5-10 wt%, glycerin at 5-10 wt%, thiodiglycol at 5-10 wt%, and an acetylene alcohol at 1 wt% of the liquid.

156. The method according to claim 155 wherein the acetylene alcohol has a structure represented by the following general formula (I):



(I)

(wherein, R_1, R_2, R_3, and R_4 represent an alkyl group, each m and n represent an integral, and m=0 and n=0 or

$1 \leq m+n \leq 30$, and when $m+n=1$, m or n is 0.)

157. The method according to claim 155 wherein a concentration of the single-stranded nucleic acid probe in the liquid is 0.05-500 μM.

158. The method according to claim 157 wherein a concentration of the single-stranded nucleic acid probe in the liquid is 2-50 μM.

159. The method according to claim 155 wherein a length of the single-stranded nucleic acid probe is 2-5,000 bases.

160. The method according to claim 159 wherein a length of the single-stranded nucleic acid probe is 2-60 bases.

161. The method according to claim 141 wherein the ink jet method is a bubble jet method.

162. The method according to claim 140 wherein the probe is an oligopeptide or a polypeptide with a specific amino acid sequence.

163. The method according to claim 140 wherein the probe is a protein.

164. The method according to claim 163 wherein the protein is an antibody.

165. The method according to claim 163 wherein the protein is an enzyme.

166. The method according to claim 140 wherein the probe is an antigen.

167. The method according to claim 140 wherein the liquid is supplied so as to form independent spots in a density of 10,000 spots per square inch on the solid support.

168. The method according to claim 140 wherein the solid support has a flat surface and homogenous surface properties.

169. The method according to claim 168 wherein the liquid is supplied on the surface of the solid support so as to obtain a distance between the adjacent spots not smaller than the maximum width of the spots.

170. The method according to claim 168 wherein blocking is performed on the surface of the solid support to prevent the sample from attaching to the surface other than the spots of the surface of the solid support.

171. The method according to claim 170 wherein blocking is achieved by using bovine serum albumin.

172. The method according to claim 140 wherein the solid support is partitioned by a matrix arranged in a pattern on the surface, a plurality of wells whose bottom is the surface of the solid support exposed in the pattern are provided, and the liquid is supplied to the respective wells.

173. The method according to claim 172 wherein the solid support is optically transparent and the matrix is opaque.

174. The method according to claim 172 wherein the matrix comprises a resin.

175. The method according to claim 172 wherein the surface of the matrix is hydrophobic.

176. The method according to claim 172 wherein the bottom of the wells is hydrophilic.

177. The method according to claim 172 wherein the matrix has a thickness of 1-20 μm.

178. The method according to claim 172 wherein the wells have a maximum width of 200 μm.

179. The method according to claim 172 wherein the matrix has a width 1/2 - 2 times the maximum width of the wells.

180. A method of identifying a structure of a target substance contained in a sample comprising the steps of:

preparing a probe array provided with spots of a prob , the probe being able to bind specifically to the target substance, on a surface of a solid support;
contacting the sample to the spots; and
detecting binding between the target substance and the prob .

181. The method of identification according to claim 180 wherein the target substance is a single-stranded nucleic acid, the structure to be identified is a base sequence of the single-stranded nucleic acid as the target substance,
the probe array is provided with a plurality of spots each of which contains single-stranded nucleic acids with different base sequences on a solid support, at least one of the spots contain a single-stranded nucleic acid with a base sequence complementary to that anticipated for the single-stranded nucleic acid as the target substance, and the plurality of spots are formed by attaching a liquid containing the respective single-stranded nucleic acids on the solid support by means of an ink jet method.

182. The method of identification according to claim 181 wherein the single-stranded nucleic acid probe is a single-stranded DNA probe.

183. The method of identification according to claim 181 wherein the single-stranded nucleic acid probe is an RNA probe.

184. The method of identification according to claim 181 wherein the single-stranded nucleic acid probe is a single-stranded PNA probe.

185. The method of identification according to claim 181 wherein the surface of the solid surface and the single-stranded nucleic acid probe have a first and a second functional groups, respectively, and the functional groups react each other by contact.

186. The method of identification according to claim 181 wherein the first functional group on the surface of the solid support is a maleimido group and the second functional group of the single-stranded nucleic acid probe is a thiol (SH) group.

187. The method of identification according to claim 186 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with N-(6-maleimidocaproyloxy) succinimide.

188. The method of identification according to claim 186 wherein the solid support is a glass plate and the maleimido group is introduced by introducing an amino group on the surface of the glass plate and then reacting the amino group with succinimidyl-4-(maleimido phenyl) butyrate.

189. The method of identification according to claim 186 wherein the maleimido group is reacted with the thiol group for at least 30 minutes.

190. The method of identification according to claim 189 wherein the single-stranded nucleic acid is a single-stranded PNA probe having a thiol group on the terminus thereof and the maleimido group is reacted with the thiol group for at least 2 hours.

191. The method of identification according to claim 186 wherein the thiol group at the terminus of the single-stranded PNA probe is introduced by binding cysteine group to an N-terminus of the single-stranded PNA probe.

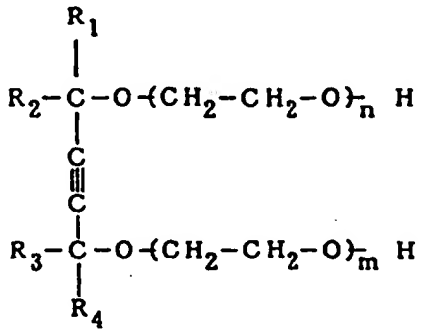
192. The method of identification according to claim 185 wherein the first functional group on the surface of the solid support is an epoxy group and the second functional group of the single-stranded nucleic acid probe is an amino group.

193. The method of identification according to claim 192 wherein the solid support is a glass plate and the epoxy group is introduced by applying a silane compound having an epoxy group in the molecule on the surface of the glass plate and reacting the compound with the glass plate.

194. The method of identification according to claim 192 wherein the epoxy group is introduced by applying polyglycidyl methacrylate having an epoxy group on the solid support.

195. The method of identification according to claim 181 wherein the liquid contains urea at 5-10 wt%, glycerin at 5-10 wt%, thiodiglycol at 5-10 wt%, and acetylene alcohol at 1 wt% of the liquid.

196. The method of identification according to claim 195 wherein the acetylene alcohol has a structure represented by the following general formula (I):



(I)

(wherein, R_1, R_2, R_3, and R_4 represent an alkyl group, each m and n represent an integral, and m=0 and n=0 or $1 \leq m+n \leq 30$, and when m+n=1, m or n is 0.)

197. The method of identification according to claim 195 wherein a concentration of the single-stranded nucleic acid probe in the liquid is 0.05-500 μM.

198. The method of identification according to claim 197 wherein the concentration of the single-stranded nucleic acid probe in the liquid is 2-50 μM.

199. The method of identification according to claim 197 wherein a length of the single-stranded nucleic acid probe is 2-5,000 bases.

200. The method of identification according to claim 199 wherein a length of the single-stranded nucleic acid probe is 2-60 bases.

201. The method of identification according to claim 181 wherein the ink jet method is a bubble jet method.

202. The method of identification according to claim 180 wherein the probe is an oligopeptide or a polypeptide with a specific amino acid sequence.

203. The method of identification according to claim 180 wherein the probe is a protein.

204. The method of identification according to claim 203 wherein the protein is an antibody.

205. The method of identification according to claim 203 wherein the protein is an enzyme.

206. The method of identification according to claim 180 wherein the probe is an antigen.

207. The method of identification according to claim 180 wherein the liquid is supplied so as to form independent spots in a density of 10,000 spots per square inch on the solid support.

208. The method of identification according to claim 180 wherein the solid support has a flat surface and homogenous surface properties.

209. The method of identification according to claim 208 wherein the liquid is supplied on the surface of the solid support so as to obtain a distance between the adjacent spots not smaller than the maximum width of the spots.

210. The method of identification according to claim 208 wherein blocking is performed on the surface of the solid

support to prevent the sample from attaching to the surface other than spots of the surfac of th solid support.

211. Th method of identification according t claim 210 wherein blocking is achieved by using bovin serum albumin.

212. Th method of identification according to claim 180 wherein the solid support is partition d by a matrix arranged in a pattern on the surface, a plurality of wells whose bottom is the surface of the solid support exposed in the pattern are provided, and the liquid is supplied to the respective wells.

213. The method of identification according to claim 212 wherein the solid support is optically transparent and the matrix is opaque.

214. The method of identification according to claim 212 wherein the matrix comprises a resin.

215. The method of identification according to claim 212 wherein the surface of the matrix is hydrophobic.

216. The method of identification according to claim 212 wherein the bottom of the wells is hydrophilic.

217. The method of identification according to claim 212 wherein the matrix has a thickness of 1-20 μm.

218. The method of identification according to claim 212 wherein the wells have a maximum width of 200 μm.

219. The method of identification according to claim 212 wherein the matrix has a width 1/2 - 2 times a maximum width of the wells.

FIG. 1

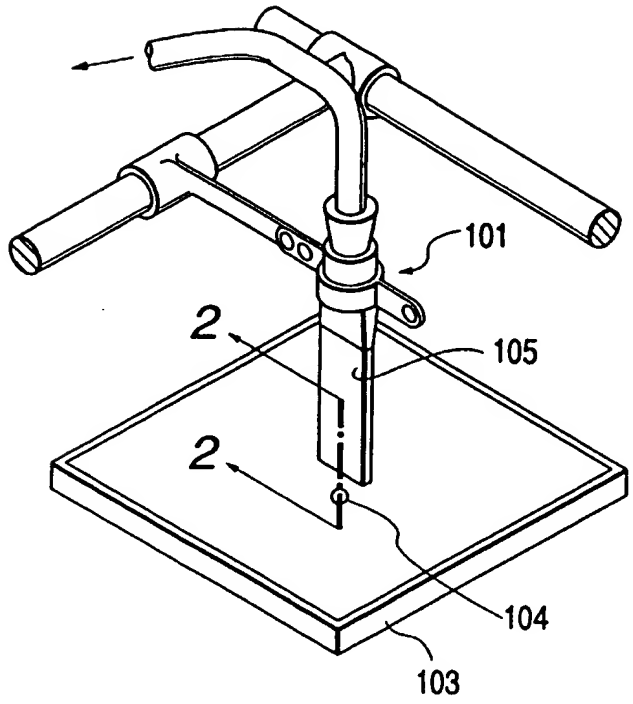

101
2
105
2
104
103

FIG. 2

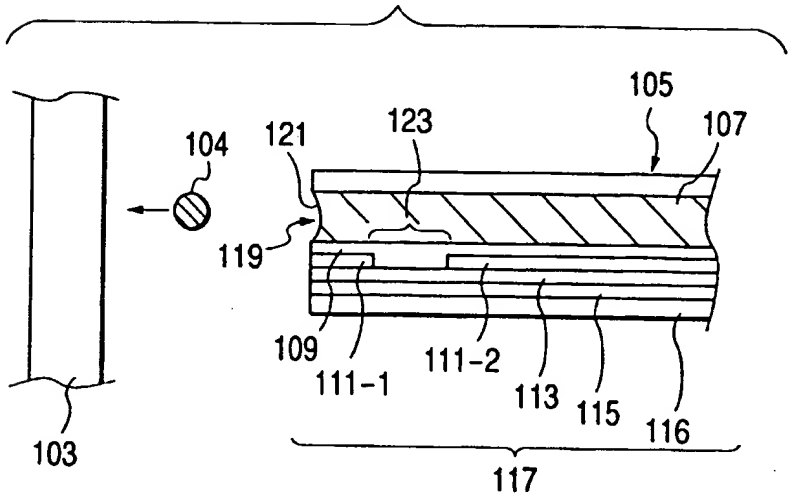

105
107
104
121
123
119
109
111-1
111-2
113
115
116
103
117

FIG. 3

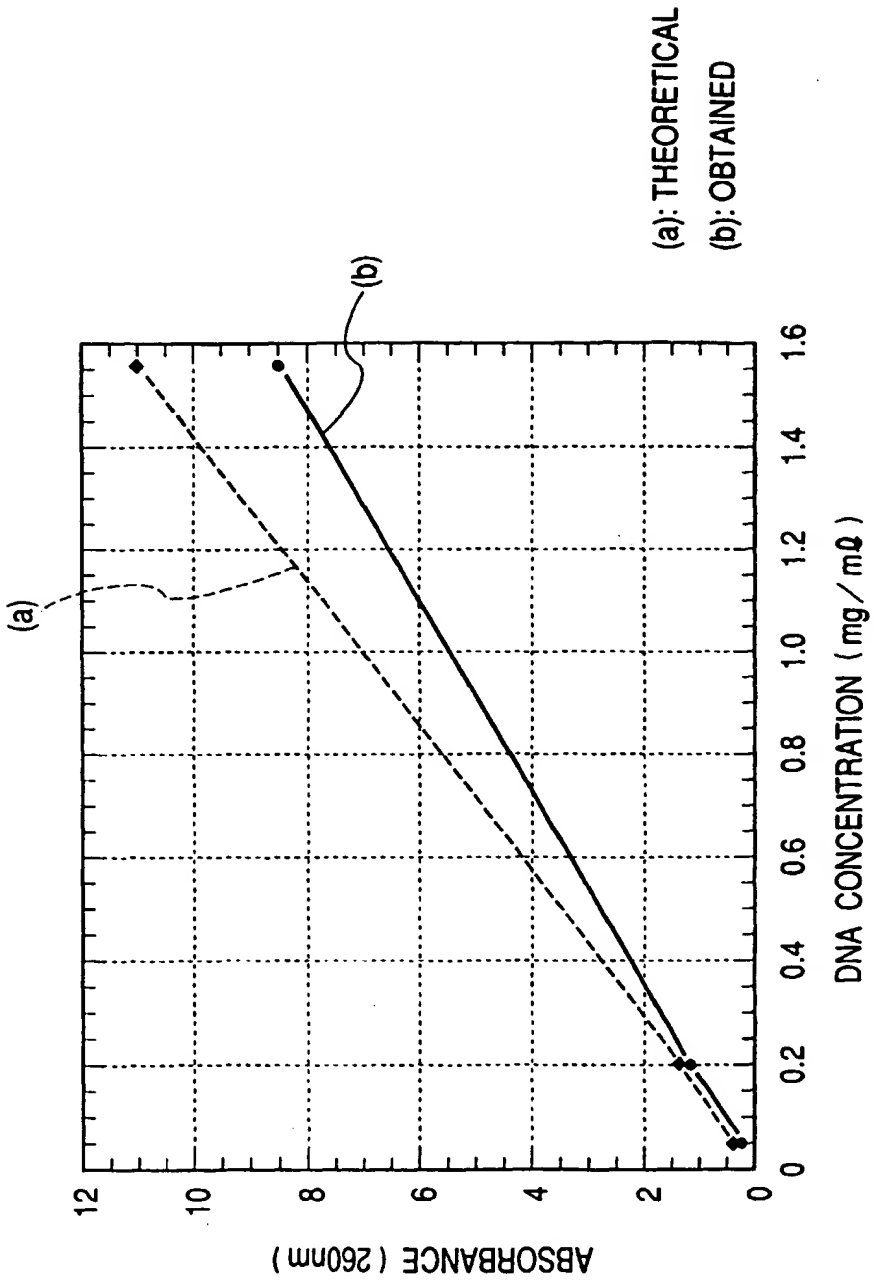

(a): THEORETICAL
(b): OBTAINED
(a)
(b)
ABSORBANCE (260nm)
0
2
4
6
8
10
12
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
DNA CONCENTRATION (mg/mℓ)

FIG. 4

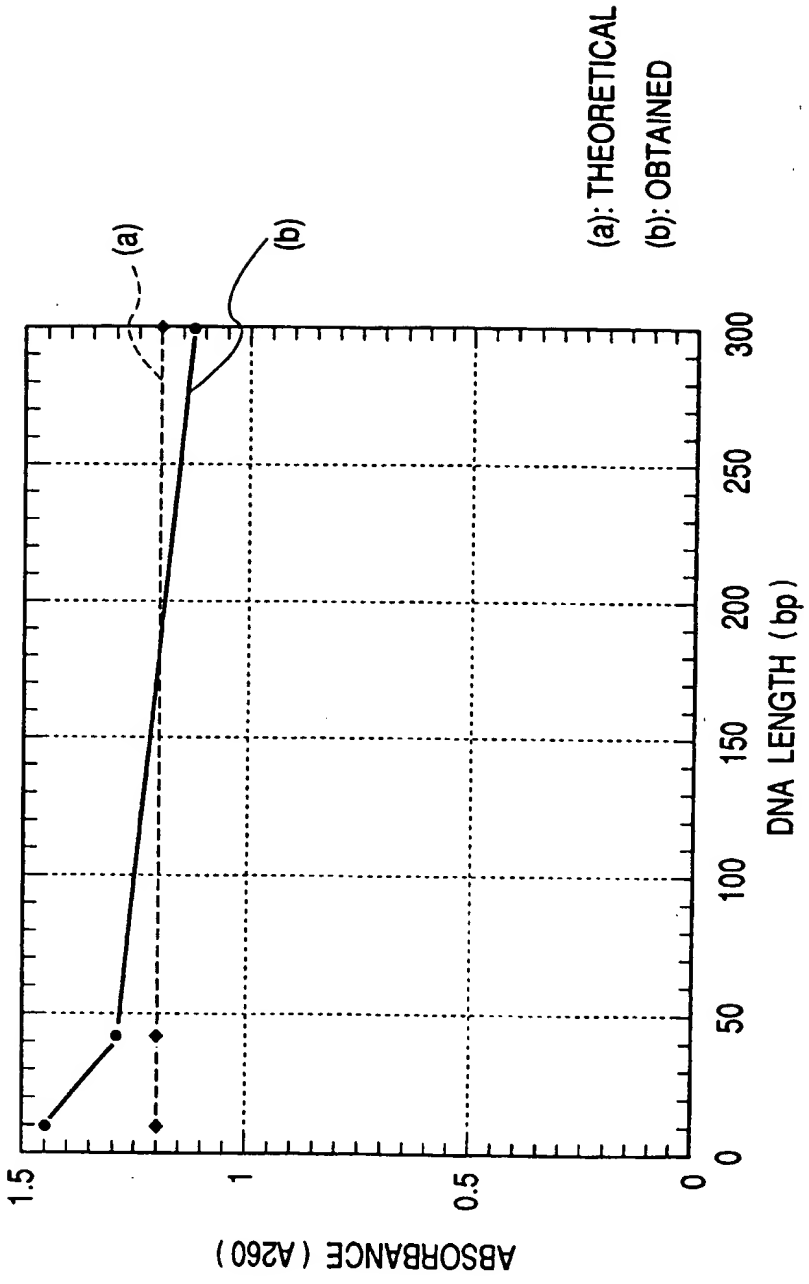

(a): THEORETICAL
(b): OBTAINED
(a)
(b)
ABSORBANCE (A260)
1.5
1
0.5
0
0
50
100
150
200
250
300
DNA LENGTH (bp)

FIG. 5A

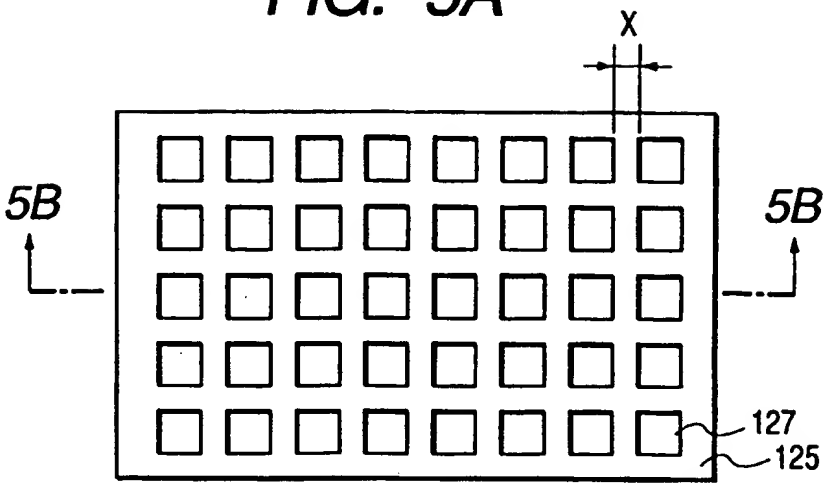


FIG. 5B

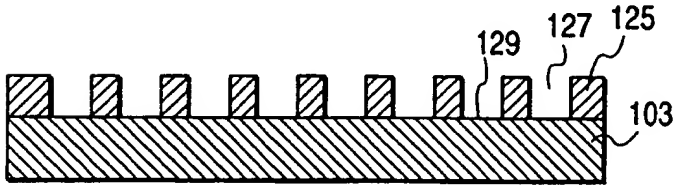


FIG. 6